International Scholarly Research Network
ISRN Pathology
Volume 2011, Article ID 953803, 10 pages
doi:10.5402/2011/953803

Research Article

Immunorecognition of the 14F7 Mab Raised against N-Glycolyl GM3 Ganglioside in Some Normal and Malignant Tissues from Genitourinary System

Rancés Blanco,[1] Mercedes Cedeño,[1] Xiomara Escobar,[2] Damián Blanco,[2] Charles E. Rengifo,[3] Milagros Frómeta,[1] Rosa Irene Alvarez,[2] Enrique Rengifo,[1] and Adriana Carr[4]

[1] *Department of Quality Control, Center of Molecular Immunology, 216 Street and 15 Avenue Atabey, Playa, P.O. Box 16040, 11600 Havana, Cuba*
[2] *Department of Cell Biology and Tissues Banking, National Institute of Oncology and Radiobiology, 29 and F Street Vedado, Plaza de la Revolución, 10400 Havana, Cuba*
[3] *Department of Pathology, Manuel Fajardo General Hospital, Zapata and D Street Vedado, Plaza de la Revolución, 10400 Havana, Cuba*
[4] *Research and Development Direction, Center of Molecular Immunology, 216 Street and 15 Avenue Atabey, Playa. P.O. Box 16040, 11600 Havana, Cuba*

Correspondence should be addressed to Rancés Blanco, rances@cim.sld.cu

Received 18 July 2011; Accepted 10 August 2011

Academic Editors: A.-J. Kruse and T. Yazawa

Copyright © 2011 Rancés Blanco et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

N-glycolyl neuraminic acid has been considered as a tumour-associated antigen forming both glycolipid and glycoprotein, expressed in some human malignant cells. In this work, we evaluate the 14F7 Mab (an IgG1 murine highly specific to N-glycolyl GM3 ganglioside) reactivity in a variety of genitourinary-system-derived tumors as well as in their normal counterparts. Immunohistochemical assays with 14F7 followed by anti-mouse biotinylated antibody and ABC/HRP system using formalin-fixed and paraffin-embedded tissues were carried out. In normal tissues, 14F7 was reactive only in renal tubules of kidney (2/6) and in the stromal component and blood vessels of ovary (3/5). Tumors of kidney (12/38), urinary bladder (8/9), breast (41/42), ovary (21/34), testis (4/5), prostate (17/20), and uterus (5/14) as well as prostatic nodular hyperplasia (5/8) were stained with 14F7. N-glycolyl GM3 recognized by 14F7 could be considered as one attractive target for both active and passive immunotherapy of genitourinary malignancies expressing this molecule.

1. Introduction

Genitourinary malignancies are formed by a group of tumors that occur in the genital and/or urinary organs. These malignant neoplasms are responsible for significant morbidity and mortality in both male and female patients [1]. Among them, breast, uterus, and prostate cancers have the highest incidence rates observed in the world [2].

Despite the availability of several options to the treatment of genitourinary system cancer, the lack of effective procedures to treat the recurrence of some of these tumors [3] has conducted to the continuous search of newer tumor-associated antigens in order to attack these molecules as a therapeutic option, alone or combined with established modalities [4, 5]. In this way, the application of immunohistochemical methods permits the selection of molecules as target for passive and/or active immunotherapy leading patients to a more appropriate therapeutic strategy [6].

Gangliosides are membrane glycosphingolipids containing one or more sialic acid residues engaged in many biological events that occur at vertebrate's cell membrane. Frequently, neoplastic cells exhibit aberrant overexpression of gangliosides present or not in normal adult tissues [7–10]. These changes allow considering some gangliosides as tumor-associated antigens [9, 11, 12]. Unusual glycolylated gangliosides have been identified by immunohistochemistry in a variety of human malignancies becoming attractive targets for immunotherapy [9, 13].

Table 1: Immunorecognition of 14F7 Mab in normal tissues from genitourinary system.

Normal tissues	No. of cases	Intensity range	Positive cells
Paraffin-embeded			
(i) Kidney	2/6	+/++	3
(a) Renal corpuscle	0/6	−	0
(b) Renal tubules	2/6	+/++	3
(ii) Bladder	0/3	−	0
(iii) Prostate	0/3	−	0
(iv) Ovary	3/5	+/++	2
(a) Epithelium	0/5	−	0
(b) Stroma	2/5	+/++	2
(c) Blood vessel	2/5	+/++	2
(v) Testis	0/2	−	0
(vi) Uterus	ND	−	−

ND: not done. Intensity: −: negative, +: weak, ++: moderate. Positive cells: 0 (negative to less than 5%), 2 (26–50%), and 3 (more than 50%).

The expression of N-glycolyl GM3 ganglioside (NeuGcGM3) in breast ductal carcinoma and the Wilms tumor using 14F7 Mab, as well as its limited presence in normal adultlinebreak human tissues, has been previously reported [9, 14]. 14F7 is the first IgG1 highly specific against NeuGcGM3 reported in the literature [9]. Here we show the evaluation of the 14F7 Mab reactivity in other benign and malignant entities of genitourinary system in both male and female patients. Additionally, samples of normal tissues were also included in the study.

2. Materials and Methods

2.1. Monoclonal Antibody. We used the 14F7 Mab (IgG1), a highly specific anti-NeuGcGM3 ganglioside antibody generated as previously described [9] and produced by the Center of Molecular Immunology (Havana, Cuba).

2.2. Tissue Specimens and Previous Processing. A number of 171 routinely processed formalin-fixed and paraffin-embedded archival samples with diagnosis of genitourinary system neoplasms were received from the Pathology Department of both the Manuel Fajardo General Hospital and the National Institute of Oncology and Radiobiology, after receiving approved consent by the institutional ethical committees. Additionally, 19 samples of normal human tissues were taken from the Legal-Medicine Department at "Amalia Simoni" Provincial Hospital of Camagüey.

Five micrometer serial sections from each block were obtained in a microtome (Lizt 1512, Germany) and mounted on Plus slides (Dako S2024, Carpinteria, USA). All sections were attached to the slide by heating at 70°C in oven for 1 h. Afterward the slides were kept at room temperature until they were used. The slides were dewaxed in xylene and rehydrated in graded ethanol series as usual and endogenous peroxidase activity was blocked with 0.03% hydrogen peroxide in methanol for 30 minutes. All sections were rehydrated in distilled water for 10 minutes and then rinsed with Tris-buffered saline (TBS).

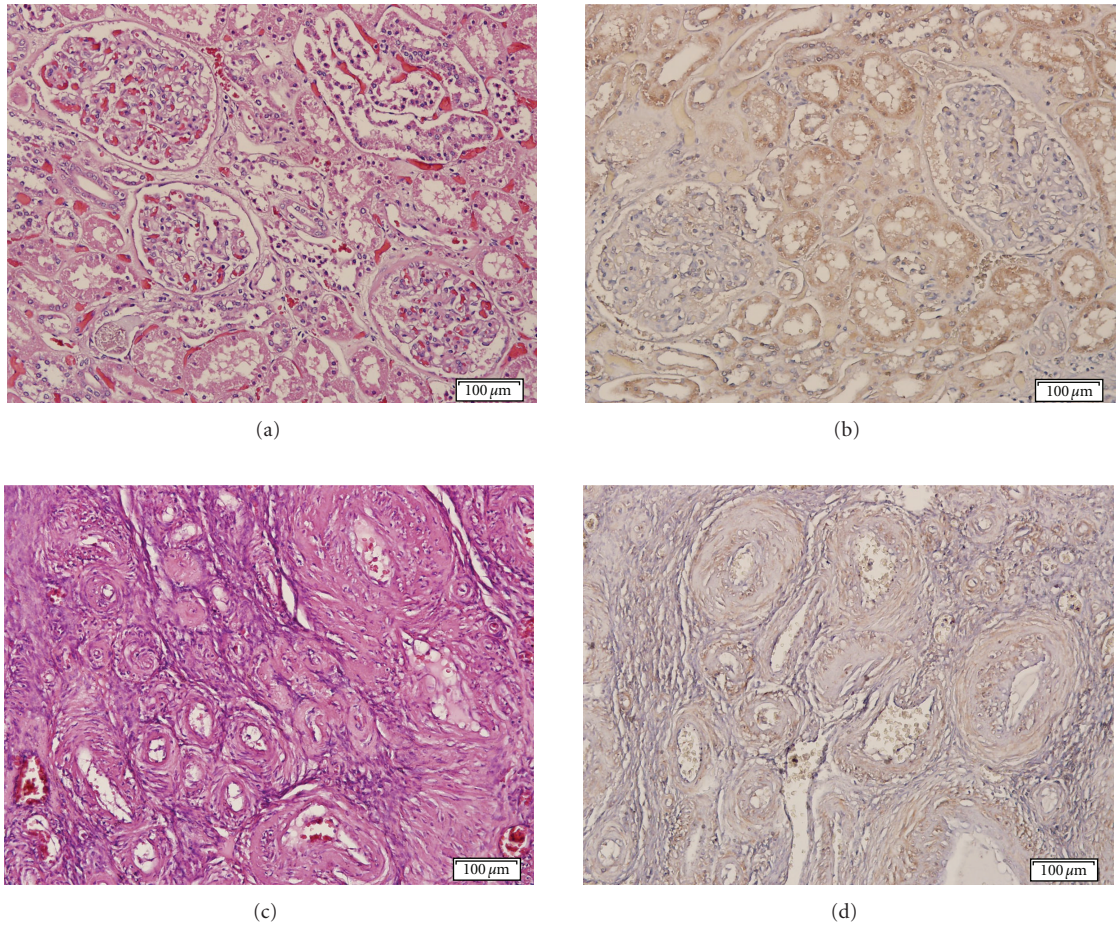
2.3. Immunohistochemical Staining. Subsequently, slides were placed in a humid chamber and incubated with the primary mouse anti-NeuGcGM3 ganglioside 14F7 Mab for 1h at room temperature. Negative controls were performed substituting primary antibody for washing buffer (TBS). Sections of colonic adenocarcinoma were taken as positive control [15].

After two rinses in TBS, the slides were incubated with a rabbit anti-mouse IgG polyclonal antibody (Dako E0354, Carpinteria, USA) diluted to 1 : 100 and with the avidin-biotin/peroxidase complex (Dako K0355, Carpinteria, USA) diluted to 1 : 100 for 30 minutes each. Between each incubation, slides were washed with TBS for 10 minutes. Enzymatic activity was visualized with DAB substrate chromogen solution (Dako K3465, Carpinteria, USA). Slides were counterstained with Mayer's Hematoxylin (Dako S2020, Carpinteria, USA), dehydrated, and mounted with a synthetic medium.

2.4. Evaluation of Results. The intensity of the reaction of each sample was qualitatively estimated and expressed as follows: negative (−), weak (+), moderate (++), and intense (+++). We used combinations of these patterns in order to express intermediate levels of immunostaining. Additionally, for each specimen, the percentage of positive tumor cells in the most representative areas was measured using a 10X magnification. Samples were scored from 0 to 3, where 0 represents the absence of immunostaining (negative up to 5%), 1 represents 6–25%, 2 represents 26–50%, and 3 represents more than 50% of the cells exhibiting staining. Results from two independent observers were considered as the final evaluation.

3. Results

3.1. Immunohistochemical Staining in Normal Tissues. The 14F7 Mab reaction in some normal tissues from genitourinary system is shown in Table 1. The reactivity of 14F7 was weak to moderate in 2/6 normal kidneys showing a cytoplasmatic pattern of staining. Both proximal and distal



(a) (b)

(c) (d)

Figure 1: Hematoxylin and eosin staining of normal kidney (a) and ovary (c) sections. Immunorecognition of 14F7 Mab ((b) and (d)). Note: a weak to moderate (finely granular) recognition was mainly located in the cytoplasm of both proximal and distal tubules, whereas no renal corpuscle reaction was evidenced (b). 14F7 also showed a weak staining in normal ovary, mainly located in the stromal and muscular component of blood vessels (d). Black bar = 100 μm.

tubules were positive, while no glomerular recognition was evidenced (Figures 1(a) and 1(b)). We also detected a weak to moderate recognition of 14F7 in 2/5 normal ovaries, mainly located in the stromal component of these tissues as well as in blood vessels (Figures 1(c) and 1(d)). No immunostaining was observed in the rest of normal tissues.

3.2. Immunohistochemical Staining in Neoplastic Tissues (I). Table 2 shows the 14F7 Mab immunorecognition in malignant and other pathological tissues derived from urinary and male genital systems.

3.2.1. Kidney. A weak to intense immunoreaction with 14F7 Mab was observed in more than 5% of neoplastic cells in 12/38 (31.58%) patients bearing renal tumors not depending on histopathological classification (Figures 2(a) and 2(b)). A finely granular staining mainly located in cell membrane of neoplastic renal cells was observed in 1/2 renal oncocytoma as well as in 7/28 clear cell renal carcinoma, 3/6 papillary renal carcinoma, and 1/1 collecting duct carcinoma. No immunostaining in a chromophobe renal carcinoma studied was evidenced.

3.2.2. Urinary Bladder. Transitional cell carcinoma of urinary bladder exhibited homogeneous and finely granular reaction with 14F7 Mab in 8/9 (88.8%) cases without taking into consideration the degree of cellular atypia gradation. The reactivity varied from moderate to intense and was mainly located in the plasmatic membrane of more than 50% of tumoral cells (data no shown).

3.2.3. Prostate. The 14F7 staining was detected on both hyperplastic glandular epithelium (62.5%) and stromal components. 14F7 Mab was moderately to intensely reactive in 4/8 (50%) of prostatic nodular hyperplasia while a weak to moderate staining was observed in 1/8 of these entities. The reactivity of 14F7 varied from weak to intense in the stromal component in 4/8 of cases.

In general, the immunorecognition of 14F7 was located mainly in plasmatic membrane and also in the cytoplasm of tumors cells in 17/20 (85%) of prostatic adenocarcinomas. Almost all moderate (3/4) and well-differentiated (11/15) tumors showed a moderate to intense reaction in more than 75% of malignant cells (Figures 2(c) and 2(d)), although a weak to moderate intensity of staining was observed

Table 2: Immunorecognition of 14F7 Mab in primary tumors of urinary and male genital systems.

Histopathological type	No. of cases (%)	Intensity range	Positive cells
Kidney	12/38 (31.6)		
Benign			
(i) Renal oncocytoma	1/2 (50)	+	3
Malignant			
(i) Renal cell carcinoma	11/36 (30.5)		
(a) Clear cell renal carcinoma	7/28 (25)	++/+++	2
(b) Papillary renal carcinoma	3/6 (50)	++/+++	3
(c) Chromophobe renal carcinoma	0/1	−	0
(d) Collecting duct carcinoma	1/1	+++	3
Bladder	8/9 (88.8)		
(i) Transitional cell carcinoma (I)	2/2	+/+++	3
(ii) Transitional cell carcinoma (II)	3/3	++/+++	3
(ii) Transitional cell carcinoma (III)	3/4 (75)	+++	3
Prostate	22/28 (78.6)		
(i) Nodular hyperplasia	5/8 (62.5)	+/++/+++	1/3
(ii) Adenocarcinoma	17/20 (85)		
(a) Well differentiated	13/15 (86.6)	+/++/+++	3
(b) Moderately differentiated	3/4 (75)	+++	3
(c) Poorly differentiated	1/1	Heterogeneous	1
Testis			
Classic seminoma	4/5 (80)	++/+++	2/3

Intensity: −: negative, ++: moderate, +++: intense. Positive cells: 0 (negative to less than 5%), 1 (6–25%), 2 (26–50%), and 3 (more than 50%).

in 2/15 of these malignancies. The poorly differentiated adenocarcinoma of this organ was reactive in less than 25% of tumor cells and showed a heterogeneous pattern. No statistically significant differences in the intensity of reaction with 14F7 (nodular hyperplasia versus adenocarcinoma) were detected ($P = 0.3396$ by chi-square test).

3.2.4. Testis. A moderate to intense, homogeneous, and finely granular immunostaining mainly located in the plasmatic membrane in more than 25% of neoplastic cells was evidenced in 4/5 (80%) of classic seminoma (Figures 2(e) and 2(f)).

3.3. Immunohistochemical Staining in Neoplastic Tissues (II). Table 3 shows the 14F7 Mab reactivity in some malignancies from female genital system.

3.3.1. Breast. A weak to intense reactivity of 14F7 Mab was observed in 41/42 (97.6%) of breast tumors, not depending on the histopathological classification. Almost all breast tumors were moderately to intensely reactive with 14F7, although, 2/12 of infiltrating ductal carcinomas (NOS), 3/10 of infiltrating lobular carcinomas, 1/10 of medullary carcinoma, and 1/2 of mucinous carcinoma showed a weak staining. The reaction was observed in more than 95% of malignant cells, mainly located in the plasmatic membrane, although a cytoplasmatic pattern was also detected (Figures 3(a) and 3(b)). Only an infiltrating lobular carcinoma was not recognized by the 14F7 Mab. No statistically significant differences (NOS versus medullary carcinoma versus lobular infiltrating carcinoma) were evidenced when the intensity of reaction was compared ($P = 0.3962$ by chi-square test).

3.3.2. Ovary. 14F7 Mab immunorecognition was evidenced in 21/34 (61.8%) of ovarian tumors showing different histopathological types. The pattern of recognition of this Mab was observed to be heterogeneous and finely granular in plasmatic membrane and cytoplasm of more than 5% of neoplastic cells in 8/12 (66.6%) serous, 7/9 (77.7%) mucinous, and 5/11 (44.5%) endometrioid adenocarcinomas as well as in 1/1 neuroendocrine tumors. No reaction with 14F7 was observed in a seromucinous adenocarcinoma (Figures 3(c) and 3(d)). When the intensity of reaction of the 14F7 Mab (serous versus mucinous versus endometrioid adenocarcinoma) was compared, statistically significant differences were observed ($P = 0.0396$ by chi-square test).

3.3.3. Uterus. Well and moderately differentiated endometrial adenocarcinomas (4/4) exhibited a moderate to intense 14F7 Mab immunoreaction located on both membrane and cytoplasm in more than 50% of neoplastic cells (Figures 3(e) and 3(f)). In contrast, poorly differentiated adenocarcinoma (0/3), cervical squamous cell carcinoma (0/4), and in *situ* carcinoma (0/2) were not reactive to 14F7.

4. Discussion

Tumour-associated aberrant glycosylation has been found in membrane glycolipids and glycoproteins as well as in

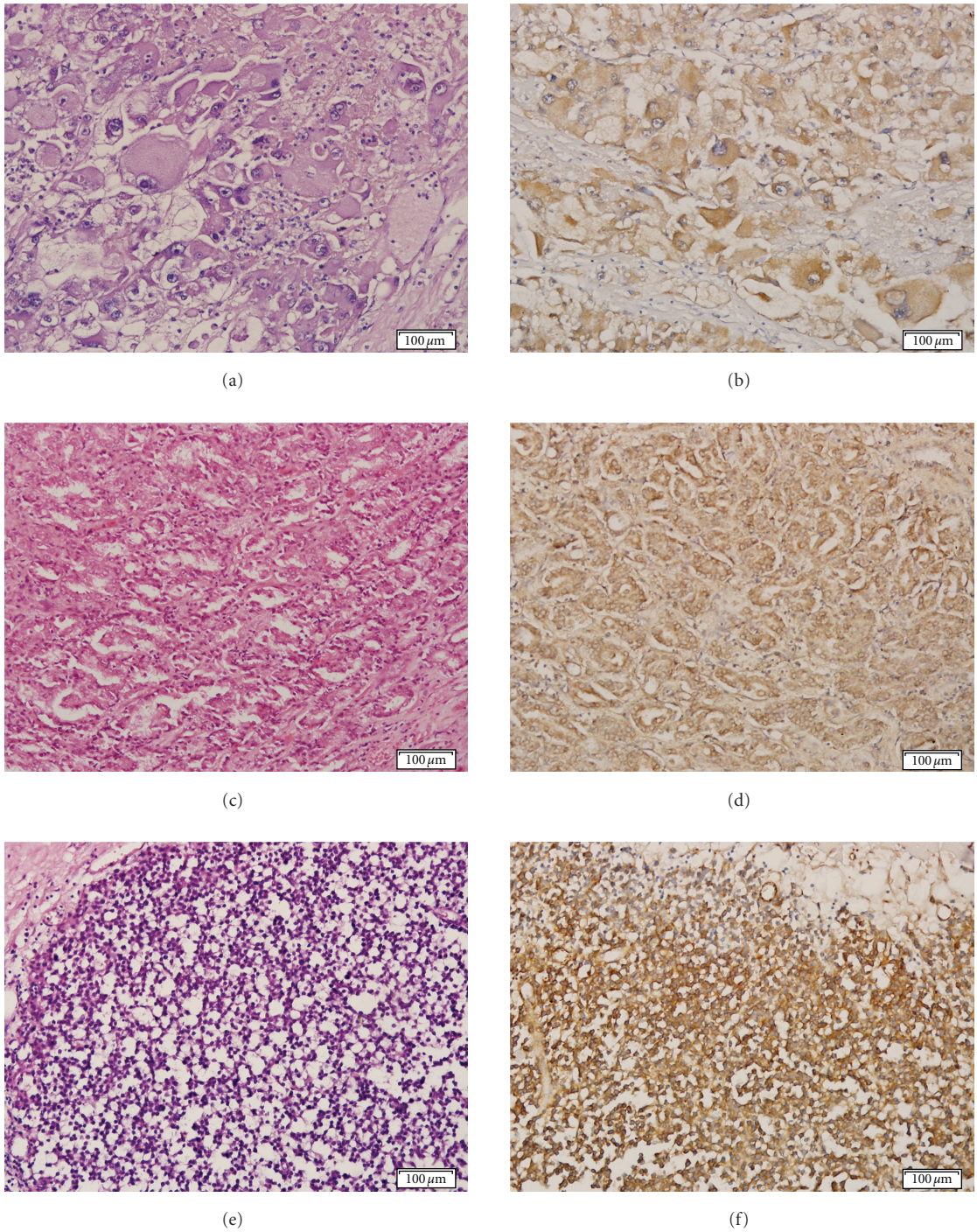


Figure 2: Hematoxylin and eosin staining of renal cell carcinoma (a), prostatic adenocarcinoma (c), and classic seminoma (e). An intense immunostaining with 14F7 Mab located on both cell membrane and cytoplasm was detected in malignant epithelial cells derived from renal tubules and prostatic glandules ((b) and (d), resp.). Note: the intense reaction of 14F7 is mainly located in cell membrane of malignant germinal cell derived from testis (f). Black bar = 100 μm.

secreted proteins [16]. Among the molecules contributing to tumor-associated carbohydrate structures, sialic acids have been considered one of the most important [17]. N-Acetyl-neuraminic (NeuAc) and N-glycolyl-neuraminic (NeuGc) are the most common sialic acids present in mammals. The structural difference between them is crucial in many aspects of cellular behavior [18, 19] and has permitted the development of specific antibodies raised against the Hanganutziu-Deicher (HD) antigen or N-glycolylated gangliosides as well as their immunohistochemical evaluation using both frozen

Table 3: Immunorecognition of 14F7 Mab in primary tumors of female genital system.

Histopathological type	No of cases (%)	Intensity range	Positive cells
Breast	41/42 (97.6)		
(i) Infiltrating ductal carcinoma	32/32 (100)		
(a) NOS	12/12	+/+++	3
(b) Papillary carcinoma	4/4	+++	3
(c) Medullary carcinoma	10/10	+/++/+++	3
(d) Metaplastic carcinoma	2/2	+++	3
(e) Mucinous carcinoma	2/2	+/++	3
(f) Tubular carcinoma	1/1	+++	3
(g) Pleomorphic carcinoma	1/1	+++	3
(ii) Infiltrating lobular carcinoma	9/10 (90)	+/++/+++	3
Ovary	22/35 (62.8)		
(i) Cystadenocarcinoma	21/34 (61.8)		
Serous	8/12 (66.6)	++/+++	3
Mucinous	7/9 (77.7)	+++	3
Seromucinous	0/1	−	0
Endometrioid	5/11 (44.5)	++/+++	1/3
(ii) Neuroendocrine tumor	1/1	++	3
Uterus	5/14 (35.7)		
(i) *Corpus* (endometrial carcinoma)	4/7 (57.1)		
Well differentiated	3/3	++/+++	3
Moderate differentiated	1/1	+++	3
Poorly differentiated	0/3	−	0
(ii) *Cervix* (carcinoma)	1/7 (14.3)		
in situ	0/2	−	0
Well differentiated	0/4	−	0
Poorly differentiated	1/1	+/++	2

NOS, Not otherwise specified. Intensity: −: negative, ++: moderate, +++: intense. Positive cells: 0 (negative to less than 5%), 1 (6–25%), 2 (26–50%), and 3 (more than 50%).

and formalin-fixed and paraffin-embedded tissues [9, 13–15, 20–22]. The antigenic determinant of HD antigen is N-glycolyl neuraminic acid [23]. Therefore, HD is classified as a heterophile antigen and chemically defined as a glycolipid and/or glycoprotein (glycoconjugates) which contains NeuGc. This antigen has been reported to be almost absent in normal human tissues, but can be expressed on a variety of human malignant cells [24].

Recently, Scursoni et al. reported the lack of reaction of 14F7 in nontumoral kidney samples from fetal autopsy. However, a cytoplasmatic reactivity of 14F7 in normal renal tubules located in peritumoral area in the Wilms tumor was detected, suggesting the shedding of gangliosides from tumor cells [14]. Here we obtained a weak to moderate staining in normal renal tubules (2/6 cases), but not in renal corpuscles. Similar results were published by Tangvoranuntakul et al. using an anti-Neu5Gc antibody. In this study, the edges of collecting duct epithelium and the associated secretions were reactive, while no glomerular staining was observed [25]. Normal eukaryotic cells are able to take in a portion of ingested Neu5Gc and process it for their own glycoconjugates [26, 27]. Afterward, the rest of NeuGc is excreted into the urine and by means other than urinary excretion [25]. This fact could support the cytoplasmatic staining of 14F7 Mab in renal tubules. We also found a weak to moderate recognition of 14F7 in normal ovary, mainly located in the stromal component, as well as in blood vessels. The reactivity of other anti-NeuGc antibodies in blood vessels has been previously reported [25].

It is known that normal cells have no metabolic pathway for NeuGc biosynthesis due to a partial deletion in the gene that encodes CMP-Neu5Ac hydroxylase [28]. The preferential aberrant expression of the NeuGc acid residues in human malignant tissues has been reported to be mainly related with its incorporation from dietary sources due to the altered and more accelerated metabolism of neoplastic cells [24, 25, 27]. Additionally, an alternative pathway to the Neu5Gc synthesis from other intermediates of cellular metabolism in some human tumors has been suggested [24].

In our study we detected a weak reactivity of 14F7 Mab in a benign renal oncocytoma while about 30% of malignant kidney tumors were moderately to intensely reactive. Mostly, tumors with clear cells and papillary differentiation patterns were recognized by 14F7. Another report of this Mab in the Wilms tumors using formalin-fixed and paraffin-embedded tissues has been recently published. Furthermore,

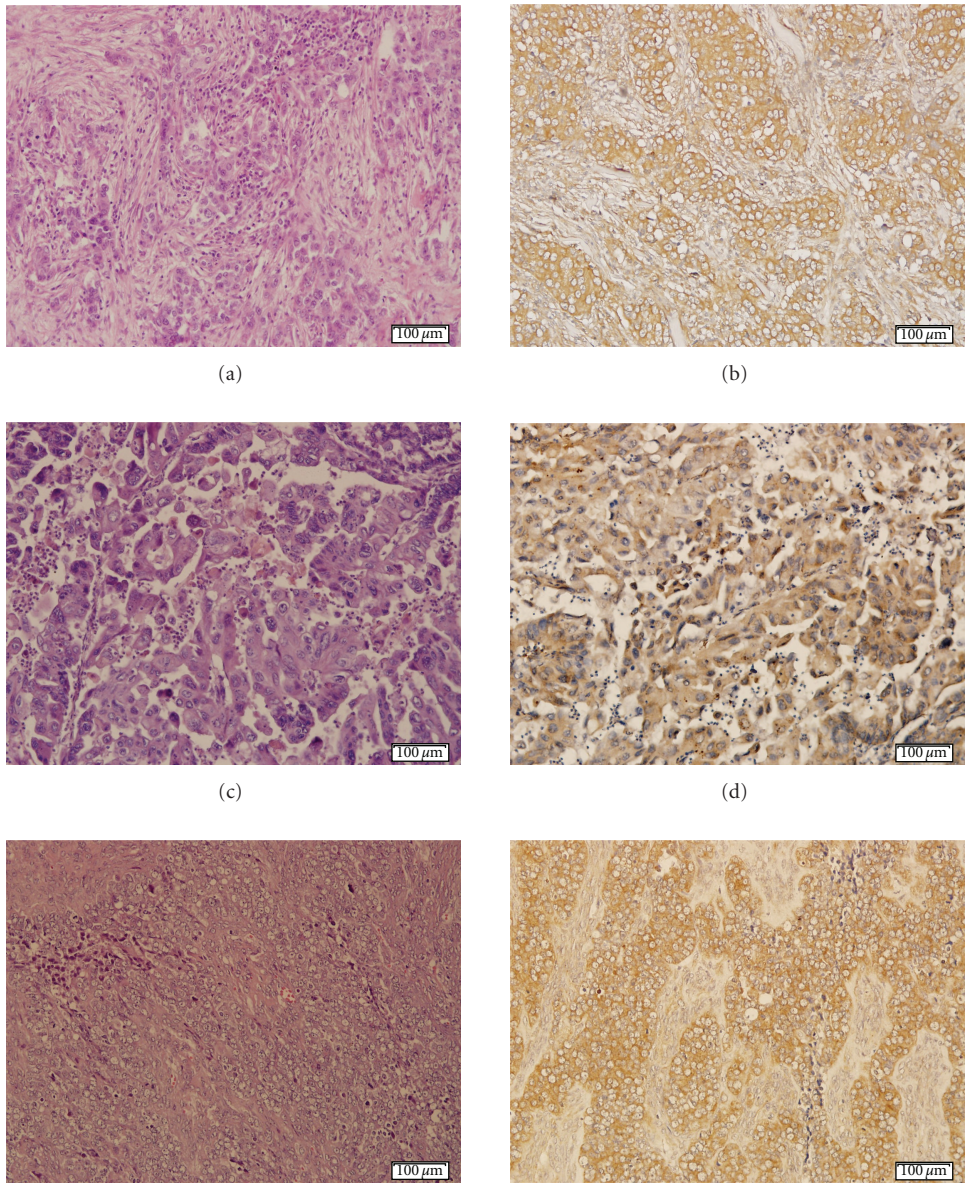


Figure 3: Hematoxylin and eosin staining of infiltrating ductal carcinoma of breast (a), well-differentiated uterine adenocarcinoma (c), and ovarian adenocarcinoma (e). Note: a strong and finely granular immunoreactivity of 14F7 Mab mainly located in cell membrane of malignant cells derived from breast and ovarian adenocarcinoma ((b) and (f), resp.). Uterine adenocarcinoma showed mainly a cytoplasmatic pattern of staining with 14F7 (d). Black bar = 100 μm.

the reaction of P3 (a specific monoclonal antibody against N-glycolylated gangliosides that also recognize sulfatides) in these malignant tumors was also reported [14].

The reactivity of some antibodies against NeuGc antigen containing glycoconjugates in breast tumors using immunohistochemical methods has been shown [13, 29, 30]. In addition, the detection of N-glycolylneuraminic acid containing ganglioside by thin-layer chromatography (TLC) immunostaining analysis using HD antibody and P3 Mab as well as the isolation of NeuGcGM3 from breast tumor tissues has been previously reported by our group [31]. Also, we published the recognition of the 14F7 Mab in breast infiltrating ductal carcinoma by immunohistochemistry using frozen tissues. Positive malignant cells showed an intense reactivity, while in normal breast tissues the immunoreaction was mainly located in the extracellular secretion but not in epithelial cells [9]. This finding suggested that the structure recognized in breast tumors could be the oligosaccharide core of NeuGcGM3 present in glycolipids, glycoproteins, or mimicries of this antigen [9]. Moreover, the ability of 14F7 Mab labelled with 99mTc to recognize breast tumors *in vivo* by the radioimmunoscintigrafic technique was demonstrated. This study was the confirmation of NeuGcGM3 expression in human breast primary tumors and

permitted us to visualize for the first time the recognition "*in vivo*" of 14F7 [32].

Here, we described the reactivity of 14F7 Mab (anti-N-glycolyl GM3 ganglioside) in formalin-fixed and paraffin-embedded breast tumors. In general, the 14F7 immunoreaction was observed in almost all breast carcinomas, not related with the histopathological subtype. A finely granular and homogeneous pattern of expression, mainly located in cell membrane was observed as we previously described [9], although a cytoplasmatic staining was also detected. The intracellular movement of glycosphingolipids and especially of gangliosides within the different subcellular compartments has been reported [33, 34]. In addition, some authors have suggested the transit of free Neu5Gc to endosomal/lysosomal system via pinocytosis, as well as its transportation to Golgi apparatus and into the cytosolic compartment, where Neu5Gc could be incorporated to newly synthesized glycoconjugates [25]. These results could support the cytoplasmatic staining observed with 14F7.

Preliminary studies using different fixative agents as well as some combinations of them (data no shown), and based on the chemical composition of NeuGcGM3 ganglioside, permitted us to consider that 14F7 probably cross-reacts not only with glycolipids in formalin-fixed and paraffin-embedded tissues, but also with other glycoconjugates containing NeuGc. It is known that gangliosides are partially or completely extracted from the tissues after ethanol and absolute methanol treatment [35]. However, previous reports suggest that the routine technique did not extract antigenic carbohydrate determinants of gangliosides [36].

Kamada et al. reported the inhibition of 3E1.2 (monoclonal antibody highly inhibited by free Neu5Gc but not by Neu5Ac) binding by ascites taken from a patient with advanced ovarian cancer. Also, the expression of HD-type antigens has been detected in serous cystadenocarcinoma of the ovary using antisera raised in rabbits by immunization with extracts of human ovarian cancer tissues [37]. Furthermore, the immunostaining of breast, ovarian, and prostate carcinoma using both frozen and formalin-fixed and paraffin-embedded tissues incubated with an anti-Neu5Gc antibody has been previously reported despite the extraction of glycolipids during the routine histological procedures [38]. Here, half of ovarian carcinomas and prostatic nodular hyperplasia, as well as almost all prostate carcinomas, were reactive to 14F7. Other studies regarding the expression of Neu5Gc in these tumors using an affinity-purified polyclonal monospecific anti-Neu5Gc chicken IgY antibody as well as increased amount of glycans containing Neu5Gc in ovarian and breast cancer by DMB derivatization and HPLC analysis have been previously reported [39].

In our study, we obtained no statistically significant differences in the intensity of reaction with 14F7 when nodular hyperplasia and adenocarcinoma were compared, although about half of the nodular hyperplasia was stained with this Mab. It is known that premalignant conditions, prostatic intraepithelial neoplasia (PIN), and atypical adenomatous hyperplasia (AAH) are associated with nodular hyperplasia and prostatic adenocarcinoma [40]. Our results likely agree with the assumption that 14F7 immunoreactivity is more related with the oncogenic transformation of the cells; however, 14F7 was not able to distinguish the premalignant zones in the nodular-hyperplasia-positive cases. On the other hand, we observed significant differences when the intensity of reaction of 14F7 in serous, mucinous, and endometrioid cystadenocarcinomas was compared. Serous and mucinous cystadenocarcinomas were mostly stained with 14F7. Our finding could be in relationship with the less aggressive biological behavior and higher survival rates observed in endometrioid carcinomas [40, 41]. This data validates the use of anti-NeuGcGM3 therapy in breast tumors independently of their histopathological type and also opens up the possibility of using this therapeutic option in ovarian and prostatic malignancies expressing this molecule.

Additionally, we showed a preliminary study of the 14F7 Mab recognition in urinary bladder and uterus tumors as well as in classic seminoma. We obtained an intense recognition of 14F7 in almost all classic seminoma, in well and moderately differentiated endometrial carcinomas but not in squamous cell carcinomas of the cervix. In addition, most of transitional cell carcinomas were stained with 14F7. The presence of N-glycolyl GM2 (another HD antigen containing ganglioside) in nonseminomatous germ cell tumors has been evidenced chemically by TLC immunostaining of the ganglioside fractions prepared from these tumors [12]. Also, the expression of Neu5Gc in human endometrial tumor mucin has been confirmed by mass spectrometry [30]. Although the number of cases is small, it would be interesting to extend the evaluation of NeuGc expression in these malignancies in order to assess its potential use as target for immunotherapy.

In summary, we are reporting the immunohistochemical recognition of 14F7 Mab, a highly specific antibody against NeuGcGM3 ganglioside, in sections of different nosological entities of the genitourinary system. It was also evidenced that it limited reactivity in normal human tissues. Our data suggest that NeuGcGM3 recognized by 14F7 Mab could be considered an attractive target for active and passive immunotherapy in order to confront genitourinary malignancies expressing this molecule. In this way, comparative studies that determinate the levels of 14F7 immunoreactivity against frozen tissues and formalin-fixed and paraffin-embedded counterparts as well as experiments for the evaluation of the chemical nature of the antigenic determinant recognized by 14F7 Mab have started. In addition, clinical trials with NeuGcGM3/VSSP molecular cancer vaccine in breast tumors are ongoing in our country.

5. Conclusions

The recognition of 14F7 Mab in some genitourinary malignancies as well as its limited reaction in normal sections supports the potential use of NeuGcGM3 recognized by 14F7 as a target for both active and passive immunotherapy of the malignant tumors expressing this molecule.

Conflict of Interests

The authors report no conflict of interests.

Acknowledgments

The authors want to thank Ph.D. Lourdes Roque-Navarro and M.S. Yuniel Fernández for providing the 14F7 monoclonal antibody and to M.S. Carmen Viada for the statistical analysis. They also want to express gratefulness to the Legal-Medicine Department at "Amalia Simoni" Provincial Hospital of Camagüey for supplying the samples of normal tissues included in this study and to M.S. Arlhee Díaz for the critical revision of the manuscript. Financial support was provided by the Center of Molecular Immunology.

References

[1] C. P. Evans, "Follow-up surveillance strategies for genitourinary malignancies," *Cancer*, vol. 94, no. 11, pp. 2892–2905, 2002.

[2] A. Jemal, R. Siegel, J. Xu, and E. Ward, "Cancer statistics, 2006," *Cancer Journal for Clinicians*, vol. 60, no. 5, pp. 277–300, 2010.

[3] K. L. Knutson, T. J. Curiel, L. Salazar, and M. L. Disis, "Immunologic principles and immunotherapeutic approaches in ovarian cancer," *Hematology/Oncology Clinics of North America*, vol. 17, no. 4, pp. 1051–1073, 2003.

[4] G. S. Palapattu and R. E. Reiter, "Monoclonal antibody therapy for genitourinary oncology: promise for the future," *Journal of Urology*, vol. 168, no. 6, pp. 2615–2623, 2002.

[5] Z. Dolićanin, L. Janković, and V. Katić, "Biomarkers for detection, treatment decision and prognosis of the urinary bladder cancer," *Medicine and Biology*, vol. 14, no. 1, pp. 1–5, 2007.

[6] K. H. Hammerich, G. A. Ayala, and T. M. Wheeler, "Application of immunohistochemistry to the genitourinary system (prostate, urinary bladder, testis, and kidney)," *Archives of Pathology and Laboratory Medicine*, vol. 132, no. 3, pp. 432–440, 2008.

[7] R. F. Irie and M. H. Ravindranath, "Gangliosides as target for monoclonal antibodies therapy of cancer," in *Therapeutic Monoclonal Antibodies*, C. A. K. Borrebaeck and G. W. Larrick, Eds., pp. 75–94, M. Stockom Press, New York, NY, USA, 1990.

[8] T. Yamashita, R. Wada, T. Sasaki et al., "A vital role for glycosphingolipid synthesis during development and differentiation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 16, pp. 9142–9147, 1999.

[9] A. Carr, A. Mullet, Z. Mazorra et al., "A mouse IgG1 monoclonal antibody specific for N-glycolyl GM3 ganglioside recognized breast and melanoma tumors," *Hybridoma*, vol. 19, no. 3, pp. 241–247, 2000.

[10] S. Birkle, G. Zeng, L. Gao, R. K. Yu, and J. Aubry, "Role of tumor-associated gangliosides in cancer progression," *Biochimie*, vol. 85, no. 3-4, pp. 455–463, 2003.

[11] H. Higashi, T. Sasabe, Y. Fukui, M. Maru, and S. Kato, "Detection of gangliosides as N-glycolylneuraminic acid-specific tumor-associated Hanganutziu-Deicher antigen in human retinoblastoma cells," *Japanese Journal of Cancer Research*, vol. 79, no. 8, pp. 952–956, 1988.

[12] M. Miyake, K. Hashimoto, M. Ito et al., "The abnormal occurrence and the differentiation-dependent distribution of N-acetyl and N-glycolyl species of the ganglioside GM2 in human germ cell tumors," *Cancer*, vol. 65, no. 3, pp. 499–505, 1990.

[13] A. M. Vazquez, M. Alfonso, B. Lanne et al., "Generation of a murine monoclonal antibody specific for N- glycolylneuraminic acid-containing gangliosides that also recognizes sulfated glycolipids," *Hybridoma*, vol. 14, no. 6, pp. 551–556, 1995.

[14] A. M. Scursoni, L. Galluzzo, S. Camarero et al., "Detection and characterization of N-glycolyated gangliosides in Wilms tumor by immunohistochemistry," *Pediatric and Developmental Pathology*, vol. 13, no. 1, pp. 18–23, 2010.

[15] R. Blanco, E. Rengifo, M. Cedeño, C. E. Rengifo, D. F. Alonso, and A. Carr, "Immunoreactivity of the 14F7 Mab raised against N-Glycolyl GM3 ganglioside in epithelial malignant tumors from digestive system," *ISRN Gastroenterology* , vol. 2011, Article ID 645641, 8 pages, 2011.

[16] E. Dabelsteen, "Cell surface carbohydrates as prognostic markers in human carcinomas," *Journal of Pathology*, vol. 179, no. 4, pp. 358–369, 1996.

[17] W. Peng-Hui, "Altered sialylation and sialyltransferase expression in gynecologic cancers," *Journal of Cancer Molecules*, vol. 2, no. 3, pp. 107–116, 2006.

[18] W. Schlenzka, L. Shaw, S. Kelm et al., "CMP-N-acetylneuraminic acid hydroxylase: the first cytosolic Rieske iron-sulphur protein to be described in Eukarya," *FEBS Letters*, vol. 385, no. 3, pp. 197–200, 1996.

[19] L. Shaw and R. Schauer, "The biosynthesis of N-glycolylneuraminic acid occurs by hydroxylation of the CMP-glycoside of N-acetylneuraminic acid," *Biological Chemistry Hoppe-Seyler*, vol. 369, no. 6, pp. 477–486, 1988.

[20] T. Saida, S. Ikegawa, Y. Takizawa, and S. Kawachi, "Immunohistochemical detection of heterophile Hanganutziu-Deicher (HD) antigen in human malignant melanoma," *Archives of Dermatological Research*, vol. 282, no. 3, pp. 179–182, 1990.

[21] H. Van Cruijsen, M. Ruiz, P. Van der Valk, T. D. de Gruijl, and G. Giaccone, "Tissue micro array analysis of ganglioside N-glycolyl GM3 expression and signal transducer and activator of transcription (STAT)-3 activation in relation to dendritic cell infiltration and microvessel density in non-small cell lung cancer," *BMC Cancer*, vol. 9, article 180, 2009.

[22] R. Blanco, E. Rengifo, C. E. Rengifo, M. Cedeño, M. Frómeta, and A. Carr, "Immunohistochemical reactivity of the 14F7 monoclonal antibody raised against N-glycolyl GM3 ganglioside in some benign and malignant skin neoplasms," *ISRN Dermatology*, vol. 2011, Article ID 848909, 8 pages, 2011.

[23] H. Higashi, M. Naiki, S. Matuo, and K. Okouchi, "Antigen of "serum sickness" type of heterophile antibodies in human sera: identification as gangliosides with N-glycolylneuraminic acid," *Biochemical and Biophysical Research Communications*, vol. 79, no. 2, pp. 388–395, 1977.

[24] Y. N. Malykh, R. Schauer, and L. Shaw, "N-Glycolylneuraminic acid in human tumours," *Biochimie*, vol. 83, no. 7, pp. 623–634, 2001.

[25] P. Tangvoranuntakul, P. Gagneux, S. Díaz et al., "Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 21, pp. 12045–12050, 2003.

[26] C. Oetke, S. Hinderlich, R. Brossmer, W. Reutter, M. Pawlita, and O. T. Keppler, "Evidence for efficient uptake and incorporation of sialic acid by eukaryotic cells," *European Journal of Biochemistry*, vol. 268, no. 16, pp. 4553–4561, 2001.

[27] M. Bardor, D. H. Nguyen, S. Diaz, and A. Varki, "Mechanism of uptake and incorporation of the non-human sialic acid N-glycolylneuraminic acid into human cells," *Journal of Biological Chemistry*, vol. 280, no. 6, pp. 4228–4237, 2005.

[28] A. Irie and A. Suzuki, "CMP-N-acetylneuraminic acid hydroxylase is exclusively inactive in humans," *Biochemical and Biophysical Research Communications*, vol. 248, no. 2, pp. 330–333, 1998.

[29] K. Ikuta, Y. Nishi, Y. Simizu et al., "Hanganutziu-Deicher type heterophyle antigen-positive cells in human cancer tissues demonstrated by membrane immunofluorescence," *Biken Journal*, vol. 25, no. 1, pp. 47–50, 1982.

[30] P. L. Devine, B. A. Clark, G. W. Birrell et al., "The breast tumor-associated epitope defined by monoclonal antibody 3E1.2 is an O-linked mucin carbohydrate containing N-glycolylneuraminic acid," *Cancer Research*, vol. 51, no. 21, pp. 5826–5836, 1991.

[31] G. Marquina, H. Waki, L. E. Fernandez et al., "Gangliosides expressed in human breast cancer," *Cancer Research*, vol. 56, no. 22, pp. 5165–5171, 1996.

[32] J. P. Oliva, Z. Valdés, A. Casacó et al., "Clinical evidences of GM3 (NeuGc) ganglioside expression in human breast cancer using the 14F7 monoclonal antibody labelled with 99mTc," *Breast Cancer Research and Treatment*, vol. 96, no. 2, pp. 115–121, 2006.

[33] B. K. Gillard, R. G. Clement, and D. M. Marcus, "Variations among cell lines in the synthesis of sphingolipids in de novo and recycling pathways," *Glycobiology*, vol. 8, no. 9, pp. 885–890, 1998.

[34] C. M. Gordon and K. O. Lloyd, "Endocytosis and recycling of gangliosides in a human melanoma cell line: inhibitory effect of brefeldin A and monensin," *Archives of Biochemistry and Biophysics*, vol. 315, no. 2, pp. 339–344, 1994.

[35] A. Schwars and A. H. Futerman, "Determination of the localization of gangliosides using anti-gangliosides antibodies: comparison of fixation methods," *Journal of Histochemistry and Cytochemistry*, vol. 45, no. 4, pp. 611–618, 1997.

[36] D. F. Alonso, M. R. Gabri, M. D. Guthmann, L. Fainboim, and D. E. Gomez, "A novel hydrophobized GM3 ganglioside/Neisseria meningitidis outer membrane protein complex vaccine induces tumor protection in B16 murine melanoma," *International Journal of Oncology*, vol. 15, no. 1, pp. 59–66, 1999.

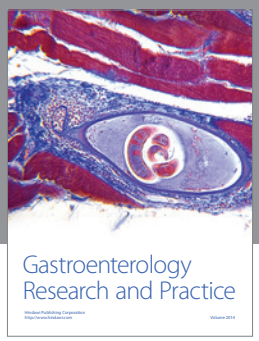
[37] M. Kamada, T. Mori, Y. Sakamoto et al., "Heterophile antigens in serous cystadenocarcinoma of the human ovary," *Asia-Oceania Journal of Obstetrics and Gynaecology*, vol. 18, no. 4, pp. 387–395, 1992.

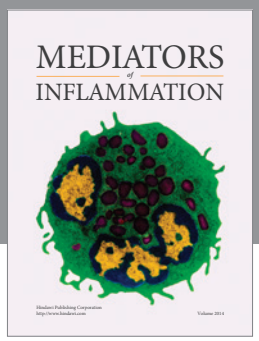
[38] S. L. Diaz, V. Padler-Karavani, D. Ghaderi et al., "Sensitive and specific detection of the non-human sialic acid N-Glycolylneuraminic acid in human tissues and biotherapeutic products," *PLoS One*, vol. 4, no. 1, article e4241, 2009.

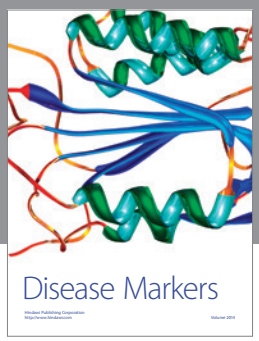
[39] M. Hedlund, V. Padler-Karavani, N. M. Varki, and A. Varki, "Evidence for a human-specific mechanism for diet and antibody-mediated inflammation in carcinoma progression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 48, pp. 18936–18941, 2008.

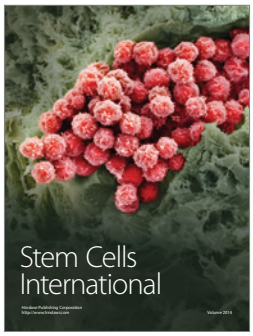
[40] B. Rekhi, T. S. Jaswal, and B. Arora, "Premalignant lesions of prostate and their association with nodular hyperplasia and carcinoma prostate," *Indian Journal of Cancer*, vol. 41, no. 2, pp. 60–65, 2004.

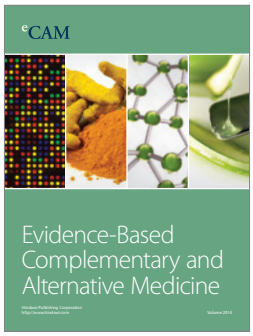
[41] C. B. Gilks and J. Prat, "Ovarian carcinoma pathology and genetics: recent advances," *Human Pathology*, vol. 40, no. 9, pp. 1213–1223, 2009.

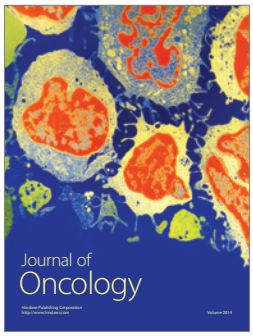


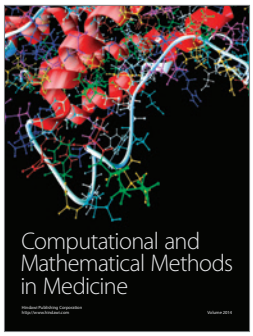


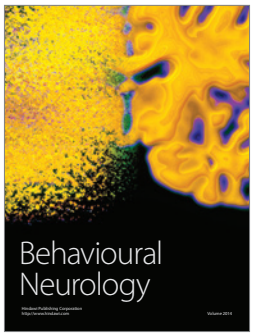


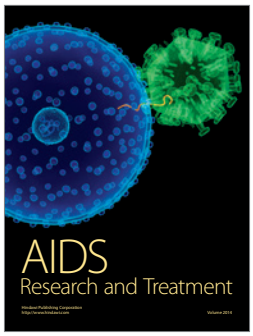


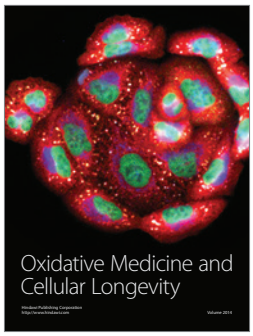












Hindawi

Submit your manuscripts at
http://www.hindawi.com